HLA Typing by SNP Genotyping

A new Method for HLA Typing at High-throughput Level

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Abstract

Allogeneic stem cell transplantation has become an effective treatment for a number of haematological diseases, such as malignant diseases (acute and chronic leukaemia, myelodysplasia), aplastic anaemia, immune-deficiencies and inherited metabolic disorders. As a matter of fact, the best donor in terms of histocompatibility is a familial HLA identical donor, e.g. HLA-identical sibling. However, most of the patients do not have a suitable familial donor and grafts from unrelated donors must be used. In recent years stem cells collected from blood and especially umbilical cord blood cells have been used, but in most of the cases the source of grafted stem cells is bone marrow. A main problem is to find a donor compatible for each patient. This is difficult to achieve due to the large genetic heterogeneity of the Major Histocompatibility Complex (MHC) immunogenetic system and its variable distribution in populations. The human MHC are the human leukocyte antigens (HLA). Large numbers of potential volunteer bone marrow donors are needed. Today more than eight million donors are registered world wide, more than two-thirds of them in 24 European registries. Improving registry efficiency is a permanent aim in order to increase the likelihood of finding compatible donors, with acceptable economic conditions. Therefore in 2001 an EU-funded project was initiated to find a new strategy to increase the efficiency paired with a reduction of the cost.

This new strategy included to perform a screen for the present HLA types of the potential donors. Therefore the alleles of the transplantation relevant HLA genes, HLA-A, HLA-B and HLA-DRB1, are divided in two groups, rare and frequent alleles. Frequent alleles are those of which the 15 most frequent HLA haplotypes are made up of. Only donors with a rare HLA type will undergo a high resolution typing procedure. To perform the HLA type screening a robust, reliable and easy-to-use method for HLA type estimation at high throughput level was required.

Within this thesis a DNA analysis-based method was developed that allows a HLA type estimation of given DNA samples at high throughput. The HLA type identification is based on microhaplotype genotyping. Microhaplotypes in this term are short DNA sequences of 4 to 5 bases length. The informativity of a microhaplotype is significantly higher then the sum of informativity of the single base polymorphisms (SNP) the microhaplotype is made up of. A set of microhaplotype markers was selected which allows a medium to high resolution

identification of the given HLA types. As the technique to genotype these microhaplotypes the GOOD assay, a purification free genotyping method with mass spectrometric detection and with a high degree of automation, was chosen.
In the framework of this thesis new software tools for microhaplotype selection and data analysis were developed, which permit a highly flexible setup of this method for other highly polymorphic regions in the genome.
After proof of principle with DNA samples from the CEPH panel, a HLA type screen at high throughput level with randomly selected DNA samples from the registries was successfully performed.